ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2010, p. 164–169 Vol. 54, No. 1
0066-4804/10/$12.00 doi:10.1128/AAC.00310-09

Transfer of Plasmid-Mediated CTX-M-9 from *Salmonella enterica* Serotype Virchow to *Enterobacteriaceae* in Human Flora-Associated Rats Treated with Cefixime[▽]

S. Faure,[1] A. Perrin-Guyomard,[1]* J. M. Delmas,[1] P. Chatre,[2] and M. Laurentie[1]

Laboratory for the Research and Investigation of Veterinary Drugs and Disinfectants, Pharmacokinetic-Pharmacodynamic Unit, AFSSA Fougères, BP 90203, La Haute Marche, 35133 Javené, France,[1] and Bacteriology Unit, AFSSA Lyon, 31, Ave. Tony Garnier, 69364 Lyon Cedex 7, France[2]

Received 6 March 2009/Returned for modification 9 May 2009/Accepted 31 October 2009

Food animals are a potential source of CTX-M resistance genes for humans. We evaluated the transfer of the $bla_{CTX\text{-}M\text{-}9}$ gene from an animal strain of *Salmonella enterica* serotype Virchow to *Enterobacteriaceae* of the human intestinal flora by using human flora-associated (HFA) rats with and without cefixime treatment. In the absence of antibiotic, no transconjugant enterobacteria were found in the feces of HFA rats. However, the transfer rate was high if *Escherichia coli* J5 recipient strains were coinoculated orally with *Salmonella*. *S. enterica* serotype Virchow persisted in the rat fecal flora both during and after treatment with therapeutic doses of cefixime. The drug did not increase the transfer rate, and *E. coli* J5 transconjugants were eliminated from the flora before the end of cefixime treatment. No cefixime was recovered in the rat feces. In the presence of recipient strains, the $bla_{CTX\text{-}M\text{-}9}$ resistance gene was transferred from a strain of animal origin to the human intestinal flora, although transconjugant colonization was transient. Antibiotic use enhanced the persistence of donor strains, increasing the resistance gene pool and the risk of its spread.

CTX-M-type extended-spectrum β-lactamases (ESBLs) are enzymes responsible for catalyzing the hydrolysis of monobactam and extended-spectrum cephalosporins. Over the last 10 years, these β-lactamases have been identified around the world, and they now have the highest prevalence of any type of ESBL (9, 10, 14, 15, 28, 32). This dramatic increase in their frequency may be attributed to the rapid dissemination of resistant bacterial clones and to their genetic structure, with the resistance genes carried on plasmids and transposons (14, 43). The consumption of contaminated food is thought to be the principal mode of spread of ESBL-producing resistant strains to the general population (31, 38). Several epidemiological analyses have investigated possible clonal relationships between resistant animal and human strains (4, 22, 25, 30, 45). Experimental studies have generated conflicting findings concerning the transfer of resistance genes in the human intestinal tract. Bonner et al. (6) suggested that bacteria from livestock cannot persist in humans and therefore do not constitute a threat. Prescott et al. (37) agreed that conditions in the human gut do not favor the transfer of resistance genes but pointed out that continuous exposure to an antibiotic would lead to the selection of organisms best able to colonize the gut (i.e., resistant strains). However, plasmid-mediated antibiotic resistance transfer may occur in the ileum (5), and Schjørring et al. (40) recently highlighted the effect of antimicrobial treatment on horizontal gene transfer from exogenous bacteria to a susceptible strain present in the mouse intestinal flora.

From 2002 to 2003, the dual emergence in France of CTX-M-9-producing multiresistant strains of *Salmonella enterica* serotype Virchow from poultry sources, and to a lesser extent from humans, was reported (45). *Salmonella* are mostly described as food-borne pathogens leading to therapeutic difficulties, especially in children, for whom extended-spectrum cephalosporins are the treatment of choice (20). Moreover, dissemination in humans of CTX-M-9 and CTX-M-14 from food has already been highlighted in 2001 in Spain (7, 36). Since CTX-M-9-producing human isolates were still rare in France in 2003, we investigated the probability of the spread of this plasmid-borne $bla_{CTX\text{-}M\text{-}9}$ gene from one strain of *S. enterica* serotype Virchow isolated from poultry to enterobacteria of the normal human intestinal flora in a human flora-associated (HFA) rat model. We also assessed the effect of cefixime on the rate of resistance transfer and the persistence of resistant strains.

MATERIALS AND METHODS

Donor and recipient strains. We studied the transfer of the plasmid-borne $bla_{CTX\text{-}M\text{-}9}$ gene from a donor strain isolated from chickens, *S. enterica* serovar Virchow strain 3464b (45). This strain was resistant to amoxicillin-clavulanic acid, cefixime, cefotaxime, ceftazidime, ceftiofur, cefuroxime, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, trimethoprim, and trimethoprim-sulfamethoxazole and has a rifampin MIC of 4 μg/ml. *Escherichia coli* J5 was used as the recipient strain (45). This strain is susceptible to cefixime and has a rifampin MIC of higher than 512 μg/ml.

Human fecal flora. We collected human fecal flora from two donors selected from seven healthy volunteers living in Brittany, France. None had received pharmacological treatment for at least 3 months before the study, and all ate traditional European meals. We checked for the absence of cefixime- and rifampin-resistant enterobacteria and *Salmonella* in each donor.

Media. Fresh human stool or HFA rat fecal samples were mixed with tryptone glucose yeast extract (AES, Bruz, France) (1%, wt/vol) under anaerobic conditions. Appropriate 10-fold serial dilutions of the supernatant were plated on selective agar media for colony counts. The compositions of the fecal aerobic and

* Corresponding author. Mailing address: Laboratory for the Research and Investigation of Veterinary Drugs and Disinfectants, Pharmacokinetic-Pharmacodynamic Unit, AFSSA Fougères, BP 90203, La Haute Marche, 35133 Javené, France. Phone: 33(2)-99-94-78-78. Fax: 33(2)-99-94-78-80. E-mail: a.perrin-guyomard@afssa.fr.

▽ Published ahead of print on 9 November 2009.

TABLE 1. Overview of the experimental design

Group	Donor strain	Recipient strain	Treatment	Cefixime dose (mg/kg)
A	*S. enterica* serotype Virchow		Water	
B	*S. enterica* serotype Virchow		Cefixime	8
C	*S. enterica* serotype Virchow	*E. coli* J5	Water	
D	*S. enterica* serotype Virchow	*E. coli* J5	Cefixime	8
E	*S. enterica* serotype Virchow	*E. coli* J5	Cefixime-clavulanic acid	8

anaerobic flora were determined as described by Perrin-Guyomard et al. (34). The total numbers of enterobacteria in the fecal flora of humans and rats were determined on Drigalski agar (Fischer-Bioblock Scientific, Illkirch, France) and compared with the counts obtained on Drigalski agar supplemented with 4 μg/ml cefixime (Sigma-Aldrich, Saint-Quentin Fallavier, France) to estimate the percentage of bacteria resistant to the antibiotic. The donor *S. enterica* serotype Virchow and recipient *E. coli* J5 strains were cultured aerobically in brain heart infusion (AES, Bruz, France). *S. enterica* serotype Virchow bacteria were counted on Brilliant green agar (Difco, BD Biosciences, Le Pont de Claix, France) supplemented with 4 μg/ml cefixime. *E. coli* J5 bacteria were counted on Drigalski agar supplemented with 250 μg/ml rifampin with the addition of 4 μg/ml cefixime for *E. coli* J5 transconjugants. All *Enterobacteriaceae*, donor, and recipient strains were incubated at 37°C for 24 h to 48 h under aerobic conditions. The detection limit for bacterial counts was 2 $\log_{10}$ CFU/g of feces. The transfer rate was defined as the number of transconjugants divided by the number of donor colonies.

Test substances. Cefixime (Oroken, 100 mg; Sanofi-Aventis, France) was administered by gavage in a dosage regimen of 4 mg/kg twice daily for 8 days, corresponding to the highest dose of cefixime that can be administered in France (41). The antibiotic was reconstituted and stored according to the manufacturer's instructions by adding sterile water to obtain a 20-g/liter stock solution and storing it at room temperature below 25°C for 1 week. The cefixime-clavulanic acid mixture (Promochem, Molsheim, France) was administered by gavage at a dose of 4 mg/kg twice daily for 8 days. The ratio of cefixime to clavulanic acid (12.5%, wt/wt) used was consistent with that commonly used in human treatment with a combination of a β-lactam and clavulanic acid (44).

Animals. All procedures for animal experiments were performed under license, with approval from the institutional review board, in accordance with French national legislation. Germ-free female and male consanguineous C3H rats (mean age, 3 weeks) from Charles River Laboratories, Arbresle, France, were transferred into a sterile Trexler-type plastic film isolator (Esi Flufrance, Massy, France) on arrival at our facilities. Each group of rats was kept in a different isolator, and each rat was housed individually in a cage, isolated from the litter by a floor grid. The germ-free status of animals was checked immediately after they were received and during the acclimatization period by testing fecal samples for the growth of aerobic and anaerobic bacteria and yeasts. Rats were provided with *ad libitum* access to a commercial diet sterilized by gamma irradiation and were supplied with sterile water.

Experimental design. HFA rats were assigned to five groups, each containing three females and two males. These groups were named A, B, C, D, and E. After 9 days of acclimatization, all germ-free rats were inoculated intragastrically with *Bacteroides fragilis* ATCC 25285 (14 $\log_{10}$ CFU/animal) to reduce oxygen and substrate concentrations in the gut. Two days later, all of the rats were inoculated intragastrically with 1 ml of a suspension of mixed human feces. After 16 days, all of the rats were inoculated intragastrically with 8 $\log_{10}$ CFU of *S. enterica* serotype Virchow (day −1).

In addition, 8 $\log_{10}$ CFU of the recipient *E. coli* J5 strain was inoculated intragastrically into the rats in groups C, D, and E 2 h before the donor strain *S. enterica* serotype Virchow. The next day (day 0), each group received antibiotic or water by gavage as follows: group A, sterile water; group B, cefixime; group C, sterile water; group D, cefixime; group E, cefixime-clavulanic acid (Table 1). Feces were collected from individual rats by provoking defecation one time before cefixime administration (day 0), five times per week during treatment, and six times after the end of treatment. Each experiment lasted 2 months.

Verification of transconjugants. On day 0, five isolates growing on Drigalski agar containing cefixime and rifampin was arbitrarily isolated from each rat sample for groups C, D, and E. Only five bacterial colonies representing the group were identified as *E. coli* by PCR amplification of the *uidA* gene, as described by Bej et al. (2, 3). DNA was prepared from samples by adding the InstaGene Matrix kit (Bio-Rad, Marnes la Coquette, France) to the bacterial suspension according to the manufacturer's instructions. The transconjugants were then characterized by pulsed-field gel electrophoresis (PFGE). PFGE analysis was performed with BlnI (Amersham Biosciences, Orsay, France) digestion in a CHEF-DRIII system (Bio-Rad, Marnes la Coquette, France). The running conditions were 6 V/cm at 14°C for 24 h with pulse times ramped from 10 s to 60 s.

PCR detection of the $bla_{CTX\text{-}M\text{-}9}$ gene. A gene conferring resistance to cefixime ($bla_{CTX\text{-}M\text{-}9}$) was detected by PCR in all transconjugants. Plasmid DNA was isolated with the QIAprep Spin Midiprep kit (Qiagen, Hilden, Germany). PCR assays using primers targeting the $bla_{CTX\text{-}M\text{-}9}$ gene were carried out as described by Weill et al. (45), except that annealing was carried out for 30 s at 60°C in each cycle. The donor and recipient strains were included as positive and negative controls.

Antimicrobial drug susceptibility testing. The donor and recipient strains and 15 transconjugants were tested for susceptibility to the antimicrobial agents amoxicillin-clavulanic acid, cefotaxime, cefoxitin, ceftazidime, ceftiofur, cefuroxime, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, trimethoprim, and trimethoprim-sulfamethoxazole by using a commercially prepared, dry panel (Trek Diagnostic Systems Ltd., East Grinstead, West Sussex, United Kingdom). Etest strips on Müller-Hinton agar (AB Biodisk, Solna, Sweden) were used to determine the MIC of cefixime. The *E. coli* ATCC 25922 strain was used for quality control (QC). Isolates were defined as susceptible or resistant in accordance with clinical breakpoints proposed by CLSI (12) or epidemiological cutoff values presented by EUCAST (www.eucast.org) for cefotaxime, ceftazidime, ceftiofur, cefoxitin, and cefuroxime.

Determination of cefixime levels in fecal samples by the LC-MS method. Cefixime concentrations in fecal samples were quantified by the liquid chromatography-mass spectrometry (LC-MS) method as previously described (29). A validation study was performed in which QC rat feces samples containing known amounts of cefixime were compared with standard curves. The interassay coefficients of variation were 10.69% and 2.67% for QC samples containing 2 and 50 mg/kg, respectively, of the drug. The intra-assay coefficients of variation were 10.69% and 1.36% for QC samples containing 2 and 50 mg/kg, respectively. The lower limit of quantification was 2.18 mg/kg.

Statistical analysis. Statistical analysis was performed with Systat 12 software (Systat Software Inc., CA). The experimental unit was the rat. The effects of treatment with cefixime and cefixime-clavulanic acid were assessed by analysis of variance with interaction. The transfer rate was calculated by dividing the number of transconjugants by the number of donor colonies for each rat. The geometric mean was calculated to represent the ratio of transconjugants to donors.

RESULTS

Implantation of the human fecal flora in a rat model. HFA rats were used to study conjugal transfer in the human gastrointestinal environment, with a complex microbiota providing the colonization barrier. Most human anaerobic and aerobic bacteria were transferred and persisted in the intestine of HFA rats after inoculation. The mean composition of the rat fecal flora was similar to that of the human fecal flora, with up to about 15 $\log_{10}$ CFU/g of feces for the total anaerobic flora, 14 $\log_{10}$ CFU/g for the *Bacteroides fragilis* group, 10 $\log_{10}$ CFU/g for lactobacilli, 11 $\log_{10}$ CFU/g for bifidobacteria, 10 $\log_{10}$ CFU/g for clostridia, 11 $\log_{10}$ CFU/g for the total aerobic flora, 10 $\log_{10}$ CFU/g for enterococci, and 7 $\log_{10}$ CFU/g for *Entero-*

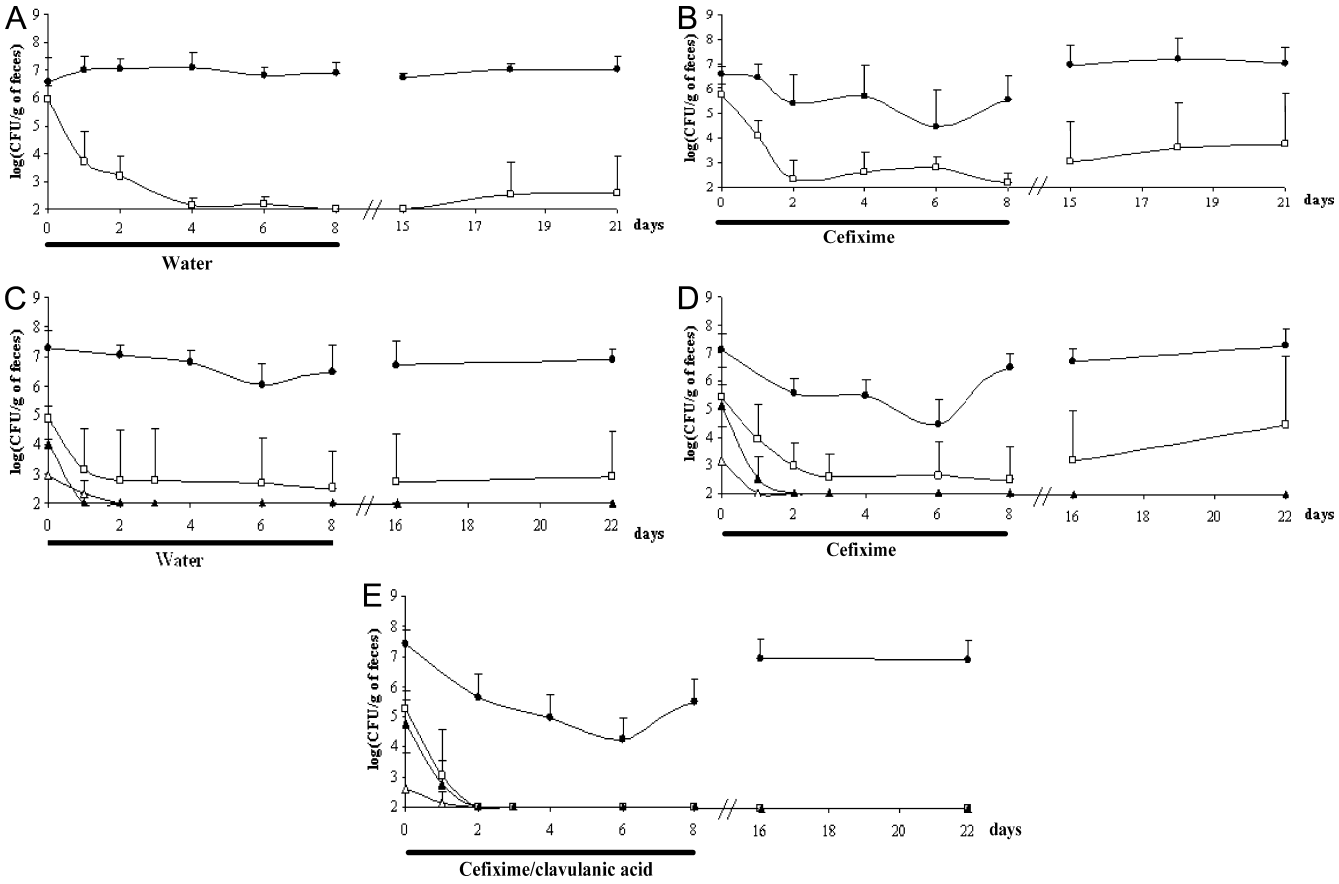

FIG. 1. Bacterial counts in the feces of HFA rats inoculated with *S. enterica* serotype Virchow (A and B) and *E. coli* J5 (C, D, and E). Animals were treated with sterile water (A and C), cefixime (B and D), or both cefixime and clavulanic acid (E). Symbols: ●, total *Enterobacteriaceae*; □, *S. enterica* serotype Virchow 3464b; ▲, *E. coli* J5; △, *E. coli* J5 transconjugants. The bar under the *x* axis represents the time of treatment. The values shown are mean results, and error bars represent standard deviations.

bacteriaceae. Before the transfer experiment, the fecal flora of HFA rats was devoid of *Salmonella* and indigenous cefixime-resistant members of the family *Enterobacteriaceae*.

Transfer of the $bla_{CTX\text{-}M\text{-}9}$ gene to the intestines of HFA rats in the absence of selective pressure (groups A and C). The *Enterobacteriaceae* populations in groups A and C remained stable at ~7 $\log_{10}$ CFU/g throughout the experiment.

In group A, the level of the donor *S. enterica* serotype Virchow reached 6 $\log_{10}$ CFU/g of feces on day 0 and decreased significantly to ~3 $\log_{10}$ CFU/g of feces after 2 days ($P < 0.001$) (Fig. 1A). Eight days later, *S. enterica* serotype Virchow was undetectable, although it was found to have persisted in one-fifth of the HFA rats at the end of the experiment. No transconjugants of endogenous *Enterobacteriaceae* were detected in the feces of any of the rats during the experiment. In group C, counts of the *E. coli* J5 recipient strain and the *S. enterica* serotype Virchow donor strain were ~4 $\log_{10}$ CFU/g and 5.5 $\log_{10}$ CFU/g of feces, respectively, on day 0 (Fig. 1C). *E. coli* J5 transconjugants appeared rapidly after the introduction of the donor strain into all of the rats (data not shown), and their level reached ~3 $\log_{10}$ CFU/g of feces on day 0. The transfer rate was estimated at about 7 ($\pm$ 1.5) $\times 10^{-1}$ transconjugants per donor and was much higher than the *in vitro* rate of 5.9 ($\pm$5.7) $\times 10^{-8}$ (19). On day 2, a significant decrease in the counts of the donor, recipient, and transconjugant strains was observed. As reported above, *S. enterica* serotype Virchow was found to have persisted in one-fifth of rats at the end of the experiment.

Effect of antibiotic exposure on $bla_{CTX\text{-}M\text{-}9}$ gene transfer (groups B, D, and E). In groups B, D, and E, the mean *Enterobacteriaceae* counts were significantly reduced, by ~2 $\log_{10}$, by treatment with cefixime or cefixime-clavulanic acid ($P < 0.001$) (Fig. 1B, D, and E). After treatment, *Enterobacteriaceae* counts returned to the initial level of about 7 $\log_{10}$ CFU/g of feces in all of the groups of rats.

In group B, no *Enterobacteriaceae* transconjugants were detected in fecal samples from rats treated with cefixime (Fig. 1B). The *Salmonella* counts in feces from all of the rats fell from about 6 $\log_{10}$ CFU/g of feces after inoculation to ~2 $\log_{10}$ CFU/g at the end of the period of drug treatment. Two weeks after the end of treatment, donor strains continued to be detected in the feces of three of the five rats and bacterial counts were significantly higher than those of the rats in the control group ($P < 0.03$) (group A). In groups D and E, *E. coli* J5 and *S. enterica* serotype Virchow counts were ~5 $\log_{10}$ CFU/g of feces on day 0 (Fig. 1D and E). At the same time point, *E. coli* J5 transconjugant levels reached about 3 $\log_{10}$ CFU/g of feces. Transfer rates in both groups were similar to that in the control

TABLE 2. Antibiotic susceptibilities of the strains used in this study

Antibiotic(s)	*E. coli* ATCC 25922	Donor *S. enterica* serotype Virchow 3464b	Recipient *E. coli* J5	*E. coli* J5 transconjugants[a]		
				Control	Cefixime	Cefixime-clavulanic acid
Amoxicillin-clavulanic acid	4[b]	8	4	16	8–32	8–16
Ampicillin	4	128	4	16	16–32	16
Cefixime	0.125	8	0.125	8	8	8
Cefotaxime	0.03	>8	0.03	0.5	0.5	0.5
Cefoxitin	4	16	4	8–32	16	16–128
Ceftazidime	0.125	8	0.125	1–4	1	1
Ceftiofur	0.25	>8	0.25	1–8	1	1
Cefuroxime	2	>32	2	16–32	8–16	16–32
Nalidixic acid	4	256	4	4	4	4
Rifampin	4	4	>512	>512	>512	>512
Streptomycin	2	128	2	2	2	2
Sulfamethoxazole	8	>512	8	4–8	4–8	4–8
Tetracycline	1	64	1	2–4	2	4
Trimethoprim	0.5	>64	0.5	0.5	0.5–2	1
Trimethoprim-sulfamethoxazole	<1	>16	<1	1	1	1

[a] Five of 15 *E. coli* J5 transconjugants isolated from animals treated with water (control), cefixime, or cefixime-clavulanic acid were tested to determine the MICs of antibiotics.
[b] MICs are expressed in μg/ml.

group, at $\sim 7 \times 10^{-1}$ transconjugants per donor. Two days after the beginning of cefixime or cefixime-clavulanic acid treatment, the recipient and transconjugant populations rapidly decreased in size, becoming undetectable. *Salmonella* counts also decreased after drug administration in the feces of all of the rats, falling below the detection threshold in the animals in group E, whereas the donor strain level was maintained at ~ 3 $\log_{10}$ CFU/g of feces in the animals in group D. Two weeks after the end of cefixime treatment, *Salmonella* were still present at countable levels in the feces of three of the five rats in this group.

Analysis of transconjugants. The 15 transconjugants isolated from the rats in groups C, D, and E were identified as *E. coli* by PCR. All had PFGE profiles identical to that of the *E. coli* J5 strain used for inoculation (data not shown). The presence of the $bla_{\text{CTX-M-9}}$ gene was confirmed by PCR in all of the isolates. The β-lactam antibiotic susceptibility of the transconjugants was higher than that observed in the parental strain (Table 2). Cotransfer of other genes conferring resistance to nalidixic acid, streptomycin, tetracycline, trimethoprim, sulfamethoxazole, or trimethoprim-sulfamethoxazole was not observed.

Determination of the cefixime fraction in fecal samples. Despite a quantification limit of 2.18 mg/kg, cefixime was not detected in fecal samples from any of the treated HFA rats.

DISCUSSION

In our HFA rat model, we detected no $bla_{\text{CTX-M-9}}$ gene transfer from *S. enterica* serotype Virchow to endogenous *Enterobacteriaceae*. However, the addition of a recipient strain to the normal flora of HFA rats, together with the donor strain of *Salmonella*, rapidly led to the appearance of transconjugants containing the $bla_{\text{CTX-M-9}}$ gene in rat feces. The administration of cefixime at therapeutic concentrations did not increase the transfer of the $bla_{\text{CTX-M-9}}$ gene between *S. enterica* serotype Virchow and *E. coli* J5, and the number of transconjugants was not found to be higher when the selective pressure was removed.

The lack of gene transfer from an exogenous strain to the indigenous flora can be related to the results of Bourgeois-Nicolaos et al. (8), who found no transfer of the *vanA* gene from *E. faecium* to *E. faecalis* in a similar HFA model. Colonization resistance of the indigenous flora toward exogenous *Salmonella* has rapidly decreased the number of donor strain bacteria, whereas for transfer to occur, large numbers of both the donor and recipient strains must be present simultaneously (27). The presence of the plasmid-free strain would almost certainly have inhibited the establishment of the plasmid-bearing strain in the control group (16). Even in the presence of an exogenous recipient strain, the transfer was transient, like that which Lester et al. (27) also observed from an *E. faecium* strain of animal origin to an *E. faecium* isolate of human origin. This likely reflects an ecological disadvantage of the transconjugants relative to the recipient strain. Such disadvantages have already been observed by Johnsen et al. (24), who, using competition experiments, demonstrated that *E. faecium* strains with newly acquired resistance are less fit than their susceptible parental strains.

Cefixime treatment did not enhance the transfer rate, whereas Duval-Iflah et al. (17) observed the establishment of transconjugants in the dominant population and the replacement of the parental recipient strain during ampicillin administration in HFA mice, even after the period of drug administration had ended. These discrepancies may result from differences in the plasmid incompatibility group and/or the adaptability to gastrointestinal conditions of the recipient strains used in our *in vivo* transfer experiment and that of Duval-Iflah et al. (13, 17, 21).

Cefixime treatment nevertheless had an effect on the persistence of *Salmonella* in treated rats in both trials. Indeed, at the end of the experiment, CTX-M-resistant strains were found to have persisted in a larger proportion of treated HFA rats (three of five) than of rats in the control group (one of five). In

the control group, the persistence of *Salmonella* may be attributed to the individual variability of animals. Previous studies on the selection and persistence of antibiotic-resistant *Salmonella* have indicated that antibiotic treatment increases the likelihood of the strain being maintained in the digestive tract (1, 33–35). As native *Enterobacteriaceae* may prevent the implantation of exogenous *Enterobacteriaceae*, the persistence of the CTX-M-producing *Salmonella* strain in our study may result from "substitution colonization" and proliferation of the strain in the digestive tract of rats in which the *Enterobacteriaceae* population had been disturbed by antibiotic treatment (23).

We detected no cefixime in HFA rat feces during treatment. However, the $>2\text{-log}_{10}$ decrease in *Enterobacteriaceae* counts during cefixime treatment in our study demonstrated that the antibiotic was present in the digestive tract of our rats. According to the pharmacokinetic study of cefixime in rats, the bioavailability of cefixime was estimated at 30% (data not shown) and comparable to the rat and human data previously described (18, 39), so the part excreted in the gastrointestinal tract may be considered approximately 70%. Based on these observations, we hypothesized that cefixime was hydrolyzed during transit by the β-lactamases-producing strains of anaerobic endogenous flora as previously described (11, 26, 42). We evaluated the impact of these β-lactamases by treating animals with cefixime together with clavulanic acid. However, cefixime was no longer recovered in the feces of rats also treated with clavulanic acid. The only effect of adding clavulanic acid was an elimination rate of *Salmonella* counts similar to that in the control group, with no regrowth when antibiotic treatment was ended. Cefixime seems to be hydrolyzed by the endogenous β-lactamases of the flora even in the presence of clavulanic acid. The combination of both drugs appeared, nevertheless, to be more inhibitory toward *Salmonella* than cefixime alone.

In summary, the lack of plasmid transfer between *S. enterica* serotype Virchow and the *Enterobacteriaceae* of HFA rats suggests that the probability of dissemination of extended-spectrum β-lactamases such as CTX-M-9 from animals to humans is very low. However, cephalosporin treatment contributes to the acquisition and overgrowth of antimicrobial-resistant pathogens in the gut, including multidrug-resistant *S. enterica* serotype Virchow, jeopardizing antibiotic treatment and constituting an important reservoir of resistance genes in the human digestive tract.

ACKNOWLEDGMENTS

We thank the animal keepers, S. Marteau and J. G. Rolland, and technical assistants P. Louapre and C. Poirier from AFSSA-Fougeres for their help. We thank also D. Meunier from AFSSA-Lyon for her involvement in this project.

This work was supported by internal funding and a grant from the Brittany region.

We have no conflict of interest to declare.

REFERENCES

1. **Adamczyk, M., and G. Jagura-Burdzy.** 2003. Spread and survival of promiscuous IncP-1 plasmids. Acta Biochim. Pol. **50:**425–453.
2. **Bej, A. K., J. L. DiCesare, L. Haff, and R. M. Atlas.** 1991. Detection of *Escherichia coli* and *Shigella* spp. in water by using the polymerase chain reaction and gene probes for *uid*. Appl. Environ. Microbiol. **57:**1013–1017.
3. **Bej, A. K., S. C. McCarty, and R. M. Atlas.** 1991. Detection of coliform bacteria and *Escherichia coli* by multiplex polymerase chain reaction: comparison with defined substrate and plating methods for water quality monitoring. Appl. Environ. Microbiol. **57:**2429–2432.
4. **Bertrand, S., F.-X. Weill, A. Cloeckaert, M. Vrints, E. Mairiaux, K. Praud, K. Dierick, C. Wildemauve, C. Godard, P. Butaye, H. Imberechts, P. A. Grimont, and J. M. Collard.** 2006. Clonal emergence of extended-spectrum ß-lactamase (CTX-M-2)-producing *Salmonella enterica* serovar Virchow isolates with reduced susceptibilities to ciprofloxacin among poultry and humans in Belgium and France (2000 to 2003). J. Clin. Microbiol. **44:**2897–2903.
5. **Blake, D., K. Hillman, D. Fenlon, and J. Low.** 2003. Transfer of antibiotic resistance between commensal and pathogenic members of the *Enterobacteriaceae* under ileal conditions. J. Appl. Microbiol. **95:**428–436.
6. **Bonner, J.** 1997. Hooked on drugs: farm animals given antibiotics need less food to grow. New Sci. **153:**24–27.
7. **Bou, G., M. Cartelle, M. Tomas, D. Canle, F. Molina, R. Moure, J. Eiros, and A. Guerrero.** 2002. Identification and broad dissemination of the CTX-M-14 beta-lactamase in different *Escherichia coli* strains in the northwest area of Spain. J. Clin. Microbiol. **40:**4030–4036.
8. **Bourgeois-Nicolaos, N., C. Moubareck, N. Mangeney, M.-J. Butel, and F. Doucet-Populaire.** 2006. Comparative study of *vanA* gene transfer from *Enterococcus faecium* to *Enterococcus faecalis* and to *Enterococcus faecium* in the intestine of mice. FEMS Microbiol. Lett. **254:**27–33.
9. **Cantón, R., T. Coque, and F. Baquero.** 2003. Multi-resistant Gram-negative bacilli: from epidemics to endemics. Infect. Dis. **16:**315–325.
10. **Cantón, R., and T. M. Coque.** 2006. The CTX-M beta-lactamase pandemic. Curr. Opin. Microbiol. **9:**466–475.
11. **Chachaty, E., C. Bourneix, S. Renard, M. Bonnay, and A. Andremont.** 1993. Shedding of *Clostridium difficile*, fecal beta-lactamase activity, and gastrointestinal symptoms in 51 volunteers treated with oral cefixime. Antimicrob. Agents Chemother. **37:**1432–1435.
12. **Clinical and Laboratory Standards Institute.** 2009. Performance standards for antimicrobial susceptibility testing; 19th informational supplement M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
13. **Coleman, W., P. Goebel, and L. Leive.** 1977. Genetic analysis of *Escherichia coli* O111:B4, a strain of medical and biochemical interest. J. Bacteriol. **130:**656–660.
14. **Coque, T. M., A. Novais, A. Carattoli, L. Poirel, J. Pitout, L. Peixe, F. Baquero, R. Canton, and P. Nordmann.** 2008. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum β-lactamase CTX-M-15. Emerg. Infect. Dis. **14:**195–200.
15. **Denton, M.** 2007. *Enterobacteriaceae*. Int. J. Antimicrob. Agents **29**(Suppl. 3)**:**S9–S22.
16. **Duval-Iflah, Y., P. Raibaud, and M. Rousseau.** 1981. Antagonisms among isogenic strains of *Escherichia coli* in the digestive tracts of gnotobiotic mice. Infect. Immun. **34:**957–969.
17. **Duval-Iflah, Y., P. Raibaud, C. Tancrede, and M. Rousseau.** 1980. R-plasmid transfer from *Serratia liquefaciens* to *Escherichia coli* in vitro and in vivo in the digestive tract of gnotobiotic mice associated with human fecal flora. Infect. Immun. **28:**981–990.
18. **Duverne, C., A. Bouten, A. Deslandes, J. Westphal, J. Trouvin, R. Farinotti, and C. Carbon.** 1992. Modification of cefixime bioavailability by nifedipine in humans: involvement of the dipeptide carrier system. Antimicrob. Agents Chemother. **36:**2462–2467.
19. **Faure, S., A. Perrin-Guyomard, J.-M. Delmas, and M. Laurentie.** 2009. Impact of therapeutic treatment with beta-lactam on transfer of the blaCTX-M-9 resistance gene from *Salmonella enterica* serovar Virchow to *Escherichia coli* in gnotobiotic rats. Appl. Environ. Microbiol. **75:**5523–5528.
20. **Galanakis, E., M. Bitsori, S. Maraki, C. Giannakopoulou, G. Samonis, and Y. Tselentis.** 2007. Invasive non-typhoidal salmonellosis in immunocompetent infants and children. Int. J. Infect. Dis. **11:**36–39.
21. **García Fernández, A., A. Cloeckaert, A. Bertini, K. Praud, B. Doublet, F.-X. Weill, and A. Carattoli.** 2007. Comparative analysis of IncHI2 plasmids carrying $bla_{\text{CTX-M-2}}$ or $bla_{\text{CTX-M-9}}$ from *Escherichia coli* and *Salmonella enterica* strains isolated from poultry and humans. Antimicrob. Agents Chemother. **51:**4177–4180.
22. **Hasman, H., D. Mevius, K. Veldman, I. Olesen, and F. M. Aarestrup.** 2005. Beta-lactamases among extended-spectrum beta-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. J. Antimicrob. Chemother. **56:**115–121.
23. **Hudault, S., J. Guignot, and A. Servin.** 2001. *Escherichia coli* strains colonising the gastrointestinal tract protect germfree mice against *Salmonella typhimurium* infection. Gut **49:**47–55.
24. **Johnsen, P. J., G. S. Simonsen, O. Olsvik, T. Midtvedt, and A. Sundsfjord.** 2002. Stability, persistence, and evolution of plasmid-encoded VanA glycopeptide resistance in enterococci in the absence of antibiotic selection in vitro and in gnotobiotic mice. Microb. Drug Resist. **8:**161–170.
25. **Lavilla, S., J. J. Gonzalez-Lopez, E. Miro, A. Dominguez, M. Llagostera, R. M. Bartolome, B. Mirelis, F. Navarro, and G. Prats.** 2008. Dissemination of extended-spectrum beta-lactamase-producing bacteria: the food-borne outbreak lesson. J. Antimicrob. Chemother. **61:**1244–1251.
26. **Léonard, F., A. Andremont, B. Leclerq, R. Labia, and C. Tancrede.** 1989. Use of beta-lactamase-producing anaerobes to prevent ceftriaxone from degrading intestinal resistance to colonization. J. Infect. Dis. **160:**274–280.
27. **Lester, C. H., N. Frimodt-Moller, T. L. Sorensen, D. L. Monnet, and A. M.**

Hammerum. 2006. In vivo transfer of the *vanA* resistance gene from an *Enterococcus faecium* isolate of animal origin to an *E. faecium* isolate of human origin in the intestines of human volunteers. Antimicrob. Agents Chemother. **50:**596–599.

28. **Mendonça, N., J. Leitao, V. Manageiro, E. Ferreira, and M. Canica.** 2007. Spread of extended-spectrum beta-lactamase CTX-M-producing *Escherichia coli* clinical isolates in community and nosocomial environments in Portugal. Antimicrob. Agents Chemother. **51:**1946–1955.
29. **Meng, F., X. Chen, Y. Zeng, and D. Zhong.** 2005. Sensitive liquid chromatography-tandem mass spectrometry method for the determination of cefixime in human plasma: application to a pharmacokinetic study. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **819:**277–282.
30. **Mesa, R. J., V. Blanc, A. R. Blanch, P. Cortes, J. J. Gonzalez, S. Lavilla, E. Miro, M. Muniesa, M. Saco, M. T. Tortola, B. Mirelis, P. Coll, M. Llagostera, G. Prats, and F. Navarro.** 2006. Extended-spectrum beta-lactamase-producing Enterobacteriaceae in different environments (humans, food, animal farms and sewage). J. Antimicrob. Chemother. **58:**211–215.
31. **Meunier, D., E. Jouy, C. Lazizzera, M. Kobisch, and J.-Y. Madec.** 2006. CTX-M-1- and CTX-M-15-type beta-lactamases in clinical *Escherichia coli* isolates recovered from food-producing animals in France. J. Antimicrob. Agents **28:**402–407.
32. **Novais, A., R. Canton, R. Moreira, L. Peixe, F. Baquero, and T. M. Coque.** 2007. Emergence and dissemination of *Enterobacteriaceae* isolates producing CTX-M-1-like enzymes in Spain are associated with IncFII (CTX-M-15) and broad-host-range (CTX-M-1, -3, and -32) plasmids. Antimicrob. Agents Chemother. **51:**796–799.
33. **Perrin-Guyomard, A., S. Cottin, D. Corpet, J. Boisseau, and J. Poul.** 2001. Evaluation of residual and therapeutic doses of tetracycline in the human-flora-associated (HFA) mice model. Regul. Toxicol. Pharmacol. **34:**125–136.
34. **Perrin-Guyomard, A., J. Poul, D. Corpet, P. Sanders, A. Fernández, and M. Bartholomew.** 2005. Impact of residual and therapeutic doses of ciprofloxacin in the human-flora-associated mice model. Regul. Toxicol. Pharmacol. **42:**151–160.
35. **Perrin-Guyomard, A., J. Poul, M. Laurentie, P. Sanders, A. Fernández, and M. Bartholomew.** 2006. Impact of ciprofloxacin in the human-flora-associated (HFA) rat model: comparison with the HFA mouse model. Regul. Toxicol. Pharmacol. **45:**66–78.
36. **Prats, G., B. Mirelis, E. Miró, F. Navarro, T. Llovet, J. Johnson, N. Camps, Á. Domínguez, and L. Salleras.** 2003. Cephalosporin-resistant Escherichia coli among summer camp attendees with salmonellosis. Emerg. Infect. Dis. **9:**1273–1280.
37. **Prescott, J. F.** 2000. Antimicrobial drugs: miracle drugs or pig feed? Adv. Pork Prod. **11:**37–45.
38. **Rodríguez-Baño, J., L. Lopez-Cerero, M. D. Navarro, P. D. de Alba, and A. Pascual.** 2008. Faecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli*: prevalence, risk factors and molecular epidemiology. J. Antimicrob. Chemother. **62:**1142–1149.
39. **Sakamoto, H., T. Hirose, and Y. Mine.** 1985. Pharmacokinetic of FK027 in rats and dogs. J. Antibiot. (Tokyo) **38:**496–504.
40. **Schjørring, S., C. Struve, and K. A. Krogfelt.** 2008. Transfer of antimicrobial resistance plasmids from *Klebsiella pneumoniae* to *Escherichia coli* in the mouse intestine. J. Antimicrob. Chemother. **62:**1086–1093.
41. **Scientific Medical Publications of France.** 2009. Dictionnaire Vidal 2009. Scientific Medical Publications of France, Inc., New York, NY.
42. **Stark, C., C. Edlund, M. Hedberg, and C. Nord.** 1995. Induction of beta-lactamase production in the anaerobic microflora by cefoxitin. Clin. Infect. Dis. **20**(Suppl. 2)**:**S350–S351.
43. **Su, L. H., C. Chu, A. Cloeckaert, and C. H. Chiu.** 2008. An epidemic of plasmids? Dissemination of extended-spectrum cephalosporinases among *Salmonella* and other *Enterobacteriaceae*. FEMS Immunol. Med. Microbiol. **52:**155–168.
44. **Trémolières, F., R. Azarian, F. Lebas, C. Mayaud, P. Carré, and J. Micolle.** 2000. Efficacy and tolerance of two oral dosages of augmentin 1 g/125 mg twice a day versus 500 mg/125 mg thrice a day in acute exacerbations of chronic bronchitis. A multicenter comparative randomized double blind study. Med. Mal. Infect. **30:**630–640.
45. **Weill, F.-X., R. Lailler, K. Praud, A. Kerouanton, L. Fabre, A. Brisabois, P. A. Grimont, and A. Cloeckaert.** 2004. Emergence of extended-spectrum-beta-lactamase (CTX-M-9)-producing multiresistant strains of *Salmonella enterica* serotype Virchow in poultry and humans in France. J. Clin. Microbiol. **42:**5767–5773.